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Transmitted herewith for filing is the patent application of:

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For: IMMUNOASSAY

Enclosed are:

- ☒ FOUR (4) sheets of drawings
- ☒ A certified copy of a JAPANESE (1) application.
- ☐ An assignment of the invention to
- ☐ A verified statement to establish small entity status under 37 CFR 1.27.
- ☒ Also enclosed: NOTICE OF PRIORITY, EXECUTED DECLARATION (3 PAGES), WHITE ADVANCE SERIAL NUMBER POSTCARD

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TITLE OF THE INVENTION:

IMMUNOASSAY

BACKGROUND OF THE INVENTION

5 Field of the Invention:

The present invention relates to a method of determining an antigen in a test specimen, and more particularly to an immunoassay which makes use of agglutination by a two-stage reaction using an insoluble
10 carrier, has high specificity and is simple and low in cost.

Description of the Background Art:

As immunoassays based on an antigen-antibody reaction, there have heretofore been known assays making
15 use of an agglutination reaction and assays making use of an antibody labeled with an enzyme for detection. In these immunoassays, the amount of an immune complex formed by a specific antigen-antibody reaction is determined either visually or as an optical change. In
20 particular, a method (hereinafter referred to as "agglutination method") of determining an antigen in a test specimen making use of an agglutination reaction or agglutination inhibition reaction based on the antigen-antibody reaction of insolubilized particles (hereinafter
25 referred to as "immobilized antibody") obtained by holding an antibody on an insoluble carrier with an antigen responsive to the antibody permits automating of

determination, and so is applied to automatic analyzers and widely spreads.

Many of the agglutination methods heretofore in use employ latex particles as the insoluble carrier, and it is known to react an immobilized antibody using (1) a polyclonal antibody, (2) a kind of monoclonal antibody or (3) two kinds of monoclonal antibodies with an objective antigen in a test specimen to form an immune aggregate, and determine the degree of the agglutination either visually or optically. Besides, there is also known (4) a method in which an objective antigen in a test specimen is adsorbed on or bound to an insoluble carrier, and an antibody responsive to the antigen is then reacted to selectively agglutinate the insoluble carrier (Japanese Patent Application Laid-Open No. 35752/1995).

However, the above-described conventional methods involve the following drawbacks. Namely, the method of (1) is the most commonly used method but involves such problems that the polyclonal antibody cross-reacts to foreign antibodies derived from a trace amount of foreign components contained in an antigen used for antibody formation and to other components similar in structure to the objective antigen because the specificity of an assay system is affected by the specificity of the polyclonal antibody used. The method of (2) can be used only for special antigens in which there are a plurality of parts (hereinafter referred to as "recognition sites") which

participate in the antigen-antibody reaction because only a kind of monoclonal antibody is used. According to the method of (3), an immune agglutinate is formed by increasing the number of recognition sites to a number
5 corresponding to the number of antibodies by using two kinds of monoclonal antibodies. However, not that a combination of any two kinds of antibodies may be used so far as they are monoclonal antibodies responsive to the same antigen, but there is a problem that a combination
10 of special two kinds of antibodies must be selected according to an object. Further, the method of (4) involves a problem that the insoluble carrier is non-specifically adsorbed on a reactor of an automatic analyzer, so that the reactor is contaminated.

15 There is also a method in which an immobilized antibody and a free antibody are used (Japanese Patent Publication No. 31227/1991). However, this method comprises, in a reaction system in which the immobilized antibody reacts to an object of determination to form an
20 optically measurable immune agglutinate, causing both antibodies (the free antibody and immobilized antibody) to compete to the object of determination, thereby inhibiting the occurrence of immune agglutination to enlarge a measuring range. Therefore, this method is
25 different in both principle and object from the present invention in which two antibodies different in form from each other are used in order to cause and increase immune

agglutination.

SUMMARY OF THE INVENTION

In view of the above-described problems, the
5 present invention has been made and has as its object the
provision of an immunoassay which makes use of
agglutination of an immobilized antibody to an object of
determination and has high specificity.

In view of the foregoing circumstances, the present
10 inventors have carried out an extensive investigation.
As a result, it has been found that two kinds of
antibodies, which respectively recognize different sites
of an objective antigen of determination, are used and
successively reacted in a state that one of them is
15 immobilized, and the other is free, thereby permitting
the achievement of an immunoassay which has high
specificity and is simple and low in cost, thus leading
to completion of the present invention.

According to the present invention, there is thus
20 provided an immunoassay comprising reacting an
immobilized antibody obtained by holding an antibody,
which recognizes a part of an objective antigen of
determination, on insoluble carrier particles with an
antigen in a test specimen, then reacting a free antibody,
25 which recognizes an antigen site different from that
recognized by the immobilized antibody, with the antigen,
and optically determining the degree of a change in

agglutination occurred by the reaction.

According to the present invention, there is also provided an immunoassay comprising reacting a free antibody, which recognizes a part of an objective antigen of determination, with an antigen in a test specimen, then reacting an immobilized antibody obtained by holding an antibody, which recognizes an antigen site different from that recognized by the free antibody, on insoluble carrier particles with the antigen, and optically determining the degree of a change in agglutination occurred by the reaction.

The immunoassays according to the present invention have advantages that they have high specificity and are simple and low in cost, and with respect to the antibodies used, insofar as one of the immobilized antibody and the free antibody has high specificity for the objective antigen of determination, the other antibody does not need to have strict specificity and may have some cross-reactivity.

The above and other objects, features, and advantages of the present invention will be readily appreciated from the preferred embodiments of the present invention, which will be described subsequently in detail with reference to the accompanying drawings.

25

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a relationship between an

absorbance and a concentration of apoprotein B when assaying the apoprotein B according to the present invention.

Fig. 2 illustrates a relationship between an
5 absorbance and a concentration of a serum amyloid A protein (SAA) when assaying the SAA according to the present invention.

Fig. 3 illustrates a relationship between an
absorbance and a concentration of a thrombin-antithrombin
10 complex (TAT) when assaying the TAT according to the present invention.

Fig. 4 illustrates a relationship between an
absorbance and a concentration of a thrombin-antithrombin
complex (TAT) when assaying the TAT according to the
15 present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As the insoluble carrier particles useful in the practice of the present invention, any known substance
20 heretofore used in assaying an antigen or antibody using an insoluble carrier may be used without any limitation. Examples thereof include organic polymeric substances, inorganic substances, cell membranes, hemocytes and microorganisms. Preferable examples of the organic
25 polymeric substances include latex particles obtained by uniformly suspending fine powder of an acrylic acid polymer, styrene polymer, methacrylic acid polymer or the

like. Examples of the inorganic substances include fine particles of silica, alumina and the like. No particular limitation is also imposed on the particle size of the insoluble carrier particles. However, the carrier
5 particles preferably have an average particle size of generally 0.05-1 μm , particularly 0.05-0.5 μm . Further, no particular limitation is also imposed on the method of immobilizing the antibody on such an insoluble carrier, and examples thereof include physical adsorption,
10 covalent bonding, immunological bonding and magnetic bonding.

No particular limitation is imposed on a liquid suspending the immobilized antibody therein. However, a buffer solution such as a phosphate buffer, glycine
15 buffer, tris buffer or Good buffer is generally used. A pH in the reaction is preferably 5-10, particularly 6-9. No particular limitation is imposed on the concentration of the immobilized antibody in a reagent finally prepared. However, the immobilized antibody is preferably at a
20 concentration of 0.1-10 mg/ml in the suspension.

The forms of the antibodies used in the present invention are two forms of an immobilized antibody and a free antibody. These antibodies may be either monoclonal antibodies or polyclonal antibodies so far as they
25 respectively recognize different sites on an objective antigen of determination. Incidentally, with respect to the antibodies used in the present invention, insofar as

one of the immobilized antibody and the free antibody has high specificity for the object of determination, the other antibody does not need to have strict specificity and may have some cross-reactivity. The antibodies may
 5 be used either singly or in any combination so far as they satisfy the above-described conditions.

No particular limitation is imposed on the objective antigen of determination in the present invention. However, examples thereof include hormones
 10 (insulin, HCG- β , growth hormone, TSH, LH, FSH, prolactin, thyroxin, triiodothyronine, gastrin, glucagon, somatostatin and the like), enzymes (elastase, amylase, protease, lipase, ribonuclease, enolase, alkaline phosphatase and the like), serum proteins (IgG, IgA, IgM,
 15 IgE, IgD, RF, SAA, SLO, macroglobulin, TBG, glycoprotein, glycolipid, apoproteins AI, AII, B, CI, CII, CIII, D, E and F, and the like), clotting-fibrinolytic factors (TAT, PIC, ATIII, APL and the like), HbA₁C, tumor-associated antigens (CEA, α -fetoprotein, ferritin, POA, CA19-9,
 20 CA125 and the like), DNA-binding protein factors, cytokines (interferon, interleukin-1, interleukin-2 and the like), various bacteria, viruses, and protozoa (fungi, streptococci, hepatitis viruses, herpes viruses, AIDS viruses, *Toxoplasma gondii*, malaria parasites, *Entamoeba*
 25 *histolytica* and the like).

The determination of the antigen in a test specimen according to the immunoassay of the present invention is

performed, for example, in the following manner. Namely,
an agglutinate is formed by a two-stage reaction in which
the antigen is reacted with the immobilized antibody, and
the free antibody is then reacted, or another two-stage
5 reaction in which the antigen is reacted with the free
antibody, and the immobilized antibody is then reacted.
The reduction in transmitted light depending on the
amount of the agglutinate can be determined by a
spectrophotometer or an automatic analyzer to measure the
10 amount of the antigen in the specimen by its checking
with a calibration curve prepared in advance, or the like.

The principle of the reaction in the present
invention is a two-stage reaction that an immobilized
antibody or a free antibody is reacted with an objective
15 antigen to capture the antigen by the antibody, and a
detectable agglutinate is formed through the free
antibody or immobilized antibody capable of coupling with
the antigen thus capture. Therefore, the immunoassay
according to the present invention is different in
20 reaction mechanism from the conventional agglutinative
immunoassay in which immobilized antibody molecules are
agglutinated each other at one stage through an objective
antigen to form an optically detectable agglutinate.
Besides, the immunoassay according to the present
25 invention is greatly different from an immunoturbidimetry
in which an antigen-antibody reaction is conducted in the
presence of an immune reaction-accelerating component

such as polyethylene glycol 6000 to optically determine the degree of immune agglutination in that no immune reaction-accelerating component is required, and the immobilized antibody is used.

5 The present invention will hereinafter be described in more detail by the following Examples. However, the present invention is not limited to these examples.

Example 1: Assay of apoprotein B

(1) Preparation of a suspension of an anti-apoprotein B
10 antibody immobilized on particles:

Added to 5 ml of a solution obtained by mixing an anti-apoprotein B monoclonal antibody at a concentration of 1.4 mg/ml with a 0.05 M glycine buffer (pH: 8.4) were 5 ml of a 2% suspension of a polystyrene latex (product
15 of Sekisui Chemical Co., Ltd.) having an average particle size of 0.2 μ m, followed by stirring at 4°C for 2 hours. A 0.05 M glycine buffer (pH: 8.4) containing 2% bovine serum albumin was then added, and the resultant mixture was stirred overnight at 4°C to prepare a suspension of
20 an anti-apoprotein B antibody immobilized on particles.

(2) Preparation of a solution of a free anti-apoprotein B antibody:

An anti-apoprotein B polyclonal antibody was mixed at a concentration of 0.2 mg/ml with a 0.05 M glycine
25 buffer (pH: 8.4) to prepare a solution of a free anti-apoprotein B antibody.

(3) Assay of apoprotein B:

After 5 μ l of a specimen solution containing apoprotein B were added to 200 μ l of the suspension of the anti-apoprotein B antibody immobilized on particles, and the resultant mixture was warmed at 37°C for 5 minutes, 200 μ l of the solution of the free anti-apoprotein B antibody were added, thereby determining the degree of change in absorbance at a wavelength of 600 nm from 1 minute to 5 minutes after stirring the mixture. The thus-obtained relationship between the absorbance and the concentration of the apoprotein B is illustrated in Fig. 1.

Comparative Example 1-1:

Assay of apoprotein B was performed in accordance with the same process in Example 1 (3) except that 200 μ l of a 0.05 M glycine buffer were used in place of the suspension of the anti-apoprotein B antibody immobilized on particles. The thus-obtained degree of change in absorbance is illustrated in Fig. 1.

Comparative Example 1-2:

Assay of apoprotein B was performed in accordance with the same process in Example 1 (3) except that 200 μ l of a 0.05 M glycine buffer were used in place of the solution of the free anti-apoprotein B antibody. The thus-obtained degree of change in absorbance is illustrated in Fig. 1.

As apparent from Fig. 1, it is understood that changes in absorbance depending on the concentration of

the apoprotein B are observed in Example 1, while no change is recognized in both Comparative Examples 1-1 and 1-2.

Example 2: Assay of a serum amyloid A protein (SAA)

- 5 (1) Preparation of a suspension of an anti-amyloid A protein antibody immobilized on particles:

Added to 5 ml of a solution obtained by mixing an anti-amyloid A protein polyclonal antibody at a concentration of 2.8 mg/ml with a 0.05 M glycine buffer (pH: 8.4) were 5 ml of a 2% suspension of a polystyrene latex (product of Sekisui Chemical Co., Ltd.) having an average particle size of 0.2 μ m, followed by stirring at 4°C for 2 hours. A 0.05 M glycine buffer (pH: 8.4) containing 2% bovine serum albumin was then added, and 15 the resultant mixture was stirred overnight at 4°C to prepare a suspension of an anti-amyloid A protein antibody immobilized on particles.

- (2) Preparation of a solution of a free anti-serum amyloid A protein antibody:

20 A C-terminal specific anti-serum amyloid A protein polyclonal antibody prepared by immunizing a rabbit with a C-terminal portion of a serum amyloid A protein was mixed at a concentration of 0.5 mg/ml with a 0.05 M glycine buffer (pH: 8.4) to prepare a solution of a free 25 anti-serum amyloid A protein antibody.

- (3) Assay of a serum amyloid A protein:

After 4 μ l of a specimen solution containing a

serum amyloid A protein were added to 240 μ l of the suspension of the anti-amyloid A protein antibody immobilized on particles, and the resultant mixture was warmed at 37°C for 5 minutes, 80 μ l of the solution of the free anti-serum amyloid A protein antibody were added, thereby determining the degree of change in absorbance at a wavelength of 600 nm from 1 minute to 5 minutes after stirring the mixture. The thus-obtained relationship between the absorbance and the concentration of the serum amyloid A protein is illustrated in Fig. 2.

Comparative Example 2-1:

Assay of a serum amyloid A protein was performed in accordance with the same process in Example 2 (3) except that 240 μ l of a 0.05 M glycine buffer were used in place of the suspension of the anti-amyloid A protein antibody immobilized on particles. The thus-obtained degree of change in absorbance is illustrated in Fig. 2.

Comparative Example 2-2:

Assay of a serum amyloid A protein was performed in accordance with the same process in Example 2 (3) except that 80 μ l of a 0.05 M glycine buffer were used in place of the solution of the free anti-serum amyloid A protein antibody. The thus-obtained degree of change in absorbance is illustrated in Fig. 2.

As apparent from Fig. 2, it is understood that changes in absorbance depending on the concentration of the serum amyloid A protein are observed in Example 2,

while no change is recognized in both Comparative Examples 2-1 and 2-2.

Example 3: Assay of thrombin-antithrombin III complex
(TAT)

- 5 (1) Preparation of a suspension of an anti-thrombin antibody immobilized on particles:

Added to 5 ml of a solution obtained by mixing an anti-thrombin monoclonal antibody at a concentration of 1.4 mg/ml with a 0.05 M glycine buffer (pH: 8.4) were 5
10 ml of a 2% suspension of a polystyrene latex (product of Sekisui Chemical Co., Ltd.) having an average particle size of 0.2 μ m, followed by stirring at 4°C for 2 hours. A 0.05 M glycine buffer (pH: 8.4) containing 2% bovine serum albumin was then added, and the resultant mixture
15 was stirred overnight at 4°C to prepare a suspension of an anti-thrombin antibody immobilized on particles.

- (2) Preparation of a solution of a free anti-antithrombin III antibody:

An anti-antithrombin III monoclonal antibody was
20 mixed at a concentration of 0.2 mg/ml with a 0.05 M glycine buffer (pH: 8.4) to prepare a solution of a free anti-antithrombin antibody.

- (3) Assay of a thrombin-antithrombin III complex:

After 20 μ l of a specimen solution containing a
25 thrombin-antithrombin III complex were added to 200 μ l of the suspension of the anti-thrombin antibody immobilized on particles, and the resultant mixture was warmed at

37°C for 5 minutes, 100 µl of the solution of the free anti-antithrombin III antibody were added, thereby determining the degree of change in absorbance at a wavelength of 600 nm from 1 minute to 5 minutes after stirring the mixture. The thus-obtained relationship between the absorbance and the concentration of the thrombin-antithrombin III complex is illustrated in Fig. 3.

Comparative Example 3-1:

Assay of a thrombin-antithrombin III complex was performed in accordance with the same process in Example 3 (3) except that 200 µl of a 0.05 M glycine buffer were used in place of the suspension of the anti-thrombin antibody immobilized on particles. The thus-obtained degree of change in absorbance is illustrated in Fig. 3.

Comparative Example 3-2:

Assay of a thrombin-antithrombin III complex was performed in accordance with the same process in Example 3 (3) except that 100 µl of a 0.05 M glycine buffer were used in place of the solution of the free anti-antithrombin III complex antibody. The thus-obtained degree of change in absorbance is illustrated in Fig. 3.

As apparent from Fig. 3, it is understood that changes in absorbance depending on the concentration of the thrombin-antithrombin III complex are observed in Example 3, while no change is recognized in both Comparative Examples 3-1 and 3-2.

Example 4: Assay of thrombin-antithrombin III complex
(TAT)

(1) Preparation of a suspension of an anti-thrombin
antibody immobilized on particles:

5 Added to 5 ml of a solution obtained by mixing an
anti-thrombin monoclonal antibody at a concentration of
1.4 mg/ml with a 0.05 M glycine buffer (pH: 8.4) were 5
ml of a 2% suspension of a polystyrene latex (product of
Sekisui Chemical Co., Ltd.) having an average particle
10 size of 0.2 μ m, followed by stirring at 4°C for 2 hours.
A 0.05 M glycine buffer (pH: 8.4) containing 2% bovine
serum albumin was then added, and the resultant mixture
was stirred overnight at 4°C to prepare a suspension of
an anti-thrombin antibody immobilized on particles.

15 (2) Preparation of a solution of a free anti-antithrombin
III antibody:

 An anti-antithrombin III monoclonal antibody was
mixed at a concentration of 0.2 mg/ml with a 0.05 M
glycine buffer (pH: 8.4) to prepare a solution of a free
20 anti-antithrombin antibody.

(3) Assay of a thrombin-antithrombin III complex:

 After 20 μ l of a specimen solution containing a
thrombin-antithrombin III complex were added to 200 μ l of
the solution of the free anti-antithrombin III antibody,
25 and the resultant mixture was warmed at 37°C for 5
minutes, 100 μ l of the suspension of the anti-thrombin
antibody immobilized on particles were added, thereby

determining the degree of change in absorbance at a wavelength of 600 nm from 1 minute to 5 minutes after stirring the mixture. The thus-obtained relationship between the absorbance and the concentration of the thrombin-antithrombin III complex is illustrated in Fig. 4.

Comparative Example 4-1:

Assay of a thrombin-antithrombin III complex was performed in accordance with the same process in Example 4 (3) except that 200 μ l of a 0.05 M glycine buffer were used in place of the solution of the free anti-antithrombin III complex antibody. The thus-obtained degree of change in absorbance is illustrated in Fig. 4.

Comparative Example 4-2:

Assay of a thrombin-antithrombin III complex was performed in accordance with the same process in Example 4 (3) except that 100 μ l of a 0.05 M glycine buffer were used in place of the suspension of the anti-thrombin antibody immobilized on particles. The thus-obtained degree of change in absorbance is illustrated in Fig. 4.

As apparent from Fig. 4, it is understood that changes in absorbance depending on the concentration of the thrombin-antithrombin III complex are observed in Example 4, while no change is recognized in both Comparative Examples 4-1 and 4-2.

WHAT IS CLAIMED IS:

1. An immunoassay comprising reacting an
immobilized antibody obtained by holding an antibody,
5 which recognizes a part of an objective antigen of
determination, on insoluble carrier particles with an
antigen in a test specimen, then reacting a free antibody,
which recognizes an antigen site different from that
recognized by the immobilized antibody, with the antigen,
10 and optically determining the degree of a change in
agglutination occurred by the reaction.

2. The immunoassay according to Claim 1, wherein
the degree of the change in agglutination is determined
15 by determining the reduction in transmitted light
depending on the amount of an agglutinate by a
spectrophotometer or an automatic analyzer.

3. The immunoassay according to Claim 1 or 2,
20 wherein the amount of the antigen in the specimen is
determined by determining the degree of the change in
agglutination and then checking the value thus obtained
with a calibration curve.

25 4. An immunoassay comprising reacting a free
antibody, which recognizes a part of an objective antigen
of determination, with an antigen in a test specimen,

then reacting an immobilized antibody obtained by holding
an antibody, which recognizes an antigen site different
from that recognized by the free antibody, on insoluble
carrier particles with the antigen, and optically
5 determining the degree of a change in agglutination
occurred by the reaction.

5. The immunoassay according to Claim 4, wherein
the degree of the change in agglutination is determined
10 by determining the reduction in transmitted light
depending on the amount of an agglutinate by a
spectrophotometer or an automatic analyzer.

6. The immunoassay according to Claim 4 or 5,
15 wherein the amount of the antigen in the specimen is
determined by determining the degree of the change in
agglutination and then checking the value thus obtained
with a calibration curve.

ABSTRACT OF THE DISCLOSURE

Disclosed herein is an immunoassay comprising reacting an immobilized antibody obtained by holding an antibody, which recognizes a part of an objective antigen of determination, on insoluble carrier particles with an antigen in a test specimen, then reacting a free antibody, which recognizes an antigen site different from that recognized by the immobilized antibody, with the antigen; or reacting a free antibody, which recognizes a part of an objective antigen of determination, with an antigen in a test specimen, then reacting an immobilized antibody obtained by holding an antibody, which recognizes an antigen site different from that recognized by the free antibody, on insoluble carrier particles with the antigen, and optically determining the degree of a change in agglutination occurred by the reaction.

The immunoassay of the invention has advantages that it has high specificity and is simple and low in cost, and with respect to the antibodies used, insofar as one of the immobilized antibody and the free antibody has high specificity for the objective antigen of determination, the other antibody does not need to have strict specificity and may have some cross-reactivity.

Fig. 1

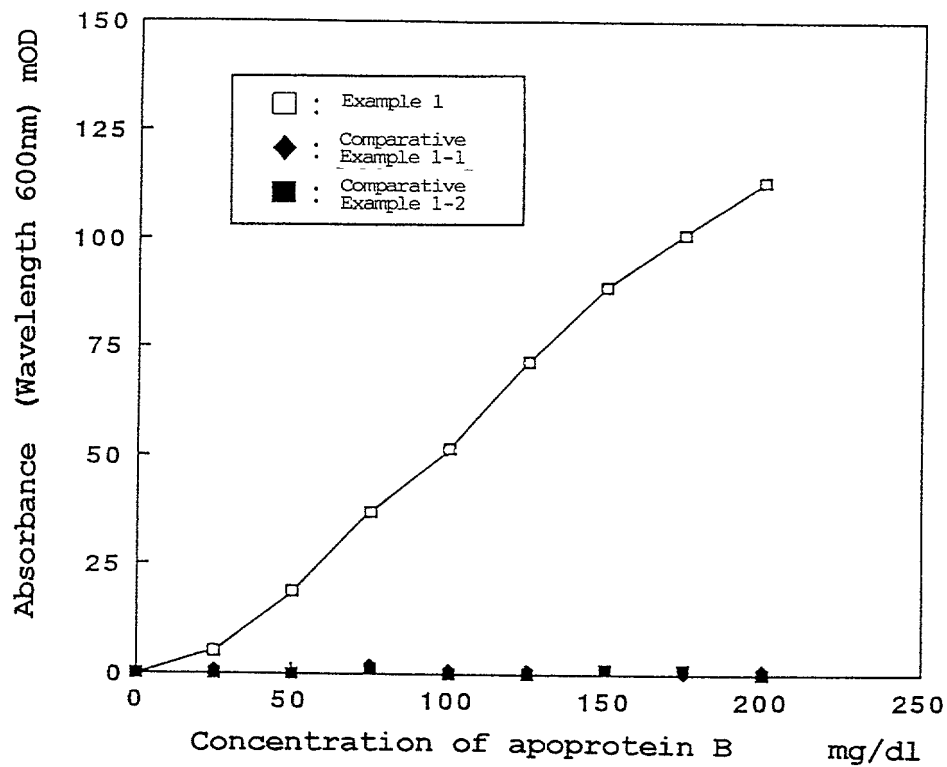


Fig. 2

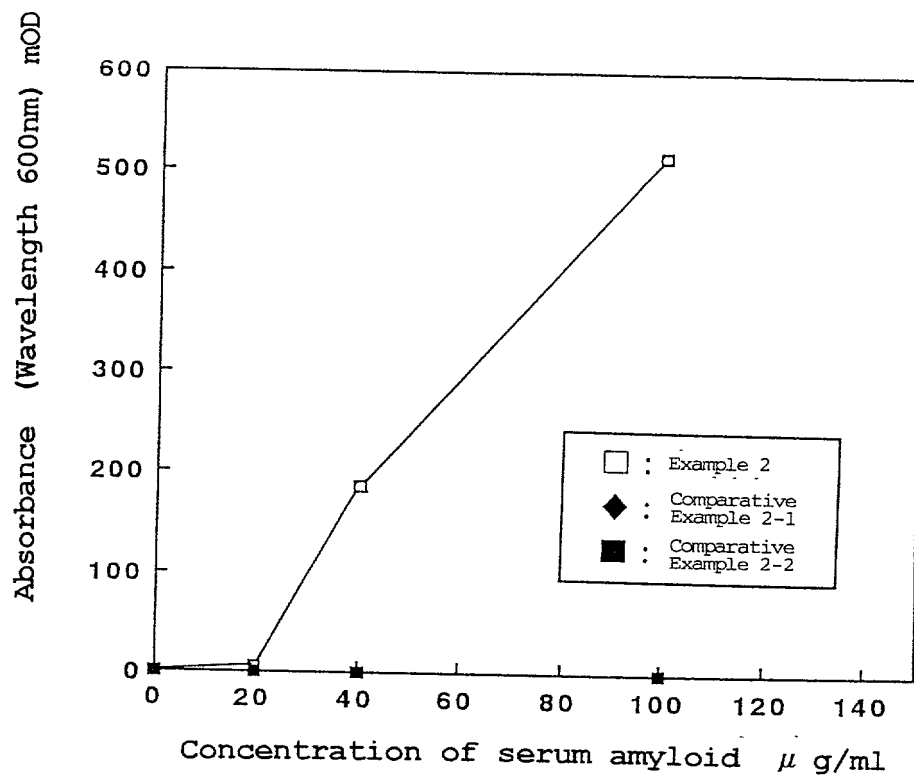


Fig. 3

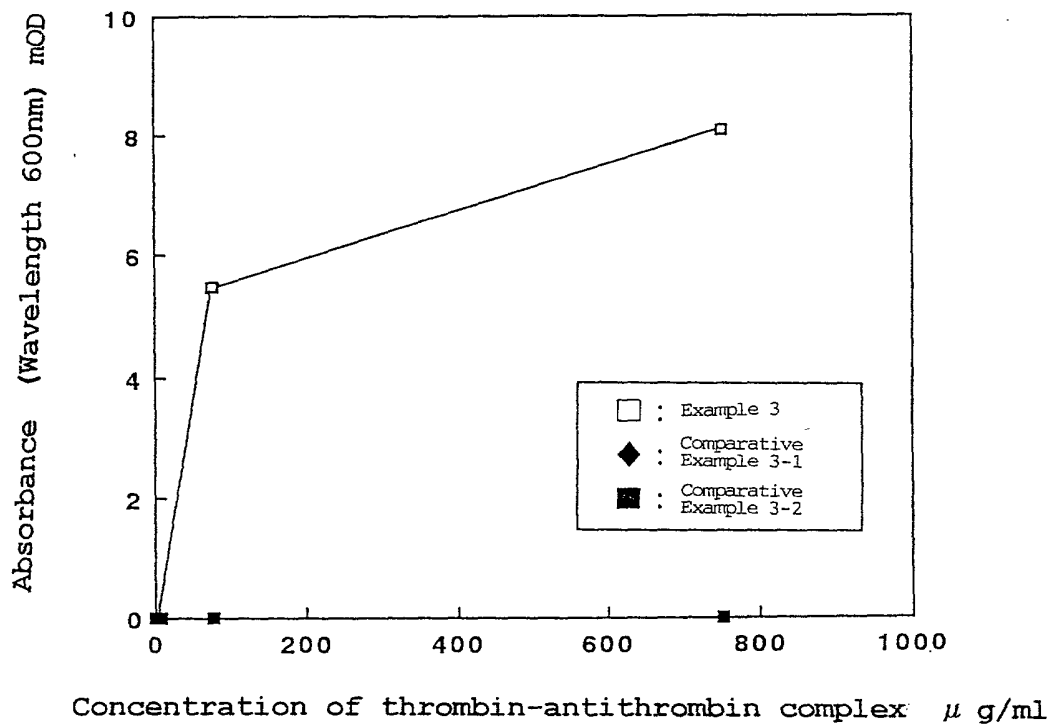
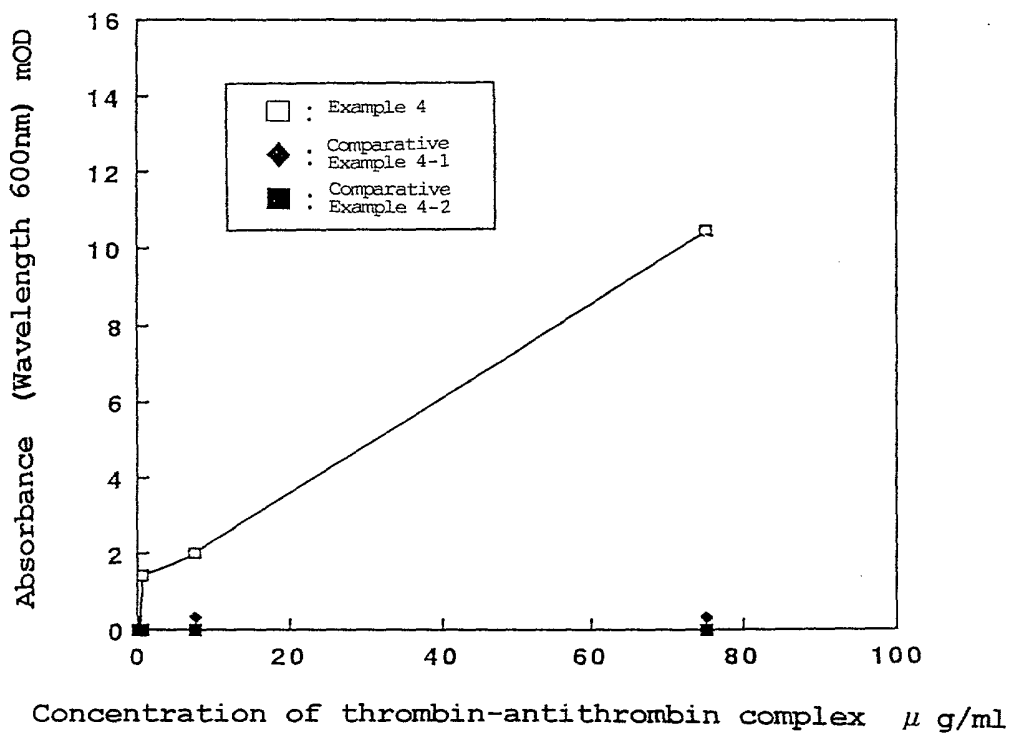


Fig. 4



Declaration, Power Of Attorney and Petition

Page 1 of 3

WE (I) the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

"IMMUNOASSAY"

the specification of which

☒ is attached hereto.

☐ was filed on _____ as

Application Serial No. _____

and amended on _____.

☐ was filed as PCT international application

Number _____

on _____,

and was amended under PCT Article 19

on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

| Application No. | Country | Day/Month/Year | Priority Claimed | |
|-----------------|---------|----------------|---|-----------------------------|
| 183279/1996 | Japan | 12/July/1996 | <input checked="" type="checkbox"/> Yes | <input type="checkbox"/> No |
| _____ | _____ | _____ | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| _____ | _____ | _____ | <input type="checkbox"/> Yes | <input type="checkbox"/> No |
| _____ | _____ | _____ | <input type="checkbox"/> Yes | <input type="checkbox"/> No |

We (I) hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

| | |
|-------------------------------|------------------------|
| _____ (Application Number) | _____ (Filing Date) |
| _____ (Application Number) | _____ (Filing Date) |

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

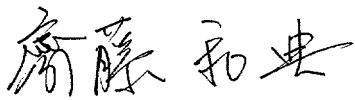
| Application Serial No. | Filing Date | Status (pending, patented, abandoned) |
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And we (I) hereby appoint: Norman F. Oblon, Registration Number 24,618; Marvin J. Spivak, Registration Number 24,913; C. Irvin McClelland, Registration Number 21,124; Gregory J. Maier, Registration Number 25,599; Arthur I. Neustadt, Registration Number 24,854; Richard D. Kelly, Registration Number 27,757; James D. Hamilton, Registration Number 28,421; Eckhard H. Kuesters, Registration Number 28,870; Robert T. Pous, Registration Number 29,099; Charles L. Gholz, Registration Number 26,395; Vincent J. Sunderdick, Registration Number 29,004; William E. Beaumont, Registration Number 30,996; Steven B. Kelber, Registration Number 30,073; Robert F. Gnuse, Registration Number 27,295; Jean-Paul Lavalle, Registration Number 31,451; Timothy R. Schwartz, Registration Number 32,171; Stephen G. Baxter, Registration Number 32,884; Martin M. Zoltick, Registration Number 35,745; Robert W. Hahl, Registration Number 33,893; Richard L. Treanor, Registration Number 36,379; Steven P. Weihrauch, Registration Number 32,829; John T. Goolkasian, Registration Number 26,142; Marc R. Labgold, Registration Number 34,651; William J. Healey, Registration Number 36,160; Richard L. Chinn, Registration Number 34,305; Steven E. Lipman, Registration Number 30,011; Carl E. Schlier, Registration Number 34,426; James J. Kulbaski, Registration Number 34,648; Catherine B. Richardson, Registration Number 39,007; Richard A. Neifeld, Registration Number 35,299; J. Derek Mason, Registration Number 35,270; and Jacques M. Dulin, Registration Number 24,067; our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to the firm of OBLON, SPIVAK, McCLELLAND, MAIER & NEUSTADT, P.C., whose Post Office Address is: Fourth Floor, 1755 Jefferson Davis Highway, Arlington, Virginia 22202.

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Kazunori SAITOH

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Signature of Inventor

June 20, 1997

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